The Restriction Requirement asserts that claims 25-73 contain twelve non-linked groups of inventions. The twelve groups asserted by the Examiner include 8 different groups drawn from claims 25-32 and 68-73, each group limited to a selection method and transformed cells comprising one enzyme in the galactose to UDP-glucose pathway: galactokinase (I), UTP-dependent phosphorylase (II), UDP-glucose-dependent uridylyltransferase (III), UDP-galactose epimerase (IV), and mutants of these (V-VIII). Claims 51-54 and 67, drawn to transformed plants, are restricted into three different groups according to the enzyme in the galactose to UDP-glucose pathway: galactokinase (IX), UTP-dependent phosphorylase (X), UDP-glucose-dependent uridylyltransferase (XI). Group XII, claims 55-66, is drawn to a transformation constructs, plasmids, kits comprising one to three nucleic acid sequences encoding enzymes that metabolize galactose, galactose precursor, or galactose derivative.

The Examiner has asserted that the instant claims are not linked by a single special technical feature "because the invention of Group I does not constitute an advance over the prior art." In support of this statement, the Examiner cites Christensen et al., for teaching selection based on the Basta herbicide and the phosphinothricin acetyltransferase gene. Applicants respectfully assert that the cited reference fails to teach or suggest the invention as claimed, and that the claims recite a specific technical feature that is not disclosed or suggested by the cited reference.

Common technical feature

Applicants submit the claims are linked by a common technical feature, that is, by the selection of transformed plant cells or tissue by the ability to survive a toxic galactose challenge, where the plant cells or tissue are transformed with a nucleic acid sequence encoding an enzyme of the galactose pathway that converts galactose to UDP-glucose. Further, Applicants assert the specific enzymes recited in the dependent claims represent particular species of the genus recited in claim 1. An election of species requirement is appropriate in this instance, rather than the multi-way restriction imposed. Rejoinder of claim groups I to IV is respectfully requested.

The claims have been amended to clarify the technical features of the invention. In particular, Applicants note the present invention is based on cells transformed with enzymes of the galactose pathway that enhance conversion of galactose to UDP-glucose and selection of

transformed cells on the basis of galactose insensitivity. This feature is demonstrated in the Working Examples as a method to identify and screen transformed cells. See, for example, Example 4 at page 60, where transformed potato shoots comprising *galT* were successfully selected on galactose.

Cells that are normally toxic to added galactose (or a galactose precursor or derivative in the galactose pathway, such as galactose-1-phosphate or UDP-galactose) are rendered galactose insensitive by expression of galactose pathway enzymes (such as galactokinase, UTP-dependent pyrophosphorylase, and UDP-glucose-dependent uridyl transferase) that are capable of enhancing the conversion of galactose to UDP-glucose, thereby reducing toxic galactose in the cell or tissue. The pathyay and enzymes are known (See the attached galactose pathway and enzyme descriptions obtained from the website of the Department of Chemistry, Queen Mary University of London, providing Enzyme Nomenclature for the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) in consultation with the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (JCBN), http://www.chem.qmw.ac.uk/iubmb/enzyme). The cited reference fails to teach or suggest use of such enzymes or galactose insensitivity as a screening method.

Christiansen does not teach the claimed invention

The Examiner cited Christensen as teaching the invention as broadly claimed in prior claim 25. Applicants respectfully submit the cited reference fails to teach or suggest selection based upon galactose toxicity in plant cells and transformation of cells with an enzyme of the galactose pathway that enhances the conversion of galactose to UDP-glucose. This requirement was present in the former pending claim 25, and is also recited in the newly presented independent claim 74.

The cited reference discloses use of the gene encoding phosphothricin acetyl transferase to confer resistance to the herbicide, Basta, as a basis for herbicide-resistance selection. This reference fails to teach use of a selection system that enhances conversion of galactose to UDP-glucose or uses galactose for selection. Applicants point to the specification, for example, at page 2, lines 1-30, where problems associated with herbicide resistance selection such as that described in Christensen is disclosed, including safety concerns for transgenic plans harboring herbicide-resistant genes. As discussed, for example, at page 3, there is a need for new selection

systems based upon metabolic advantage, such as the claimed invention, that do not pose the threat to safety that troubles herbicide resistant plants.

Christensen fails to destroy the common inventive concept linking the claims. Accordingly, Applicants assert rejoinder of the claim groups I-IV is proper, and should be made. Affirmation of rejoinder is requested.

The Examiner is invited to telephone the attorney for further discussion of the claim amendment or remarks, or to otherwise speed prosecution of this application.

Respectfully submitted,

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Date: December 4, 2002

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09/762,629 **M&G 9192.15USWO**

NEW CLAIMS **12/3/02**

74. A process for selecting transformed cells or tissue comprising:
   a) transforming plant cells or tissue that are sensitive to galactose toxicity with one or more polynucleotide molecule encoding one or more enzyme that enhances conversion of galactose to UDP-glucose;
   b) exposing the cells or tissue to galactose, wherein galactose is toxic to non-transformed cells or tissue; and
   c) selecting transformed cells or tissue that are insensitive to galactose toxicity.

75. The process of claim 74, wherein said one or more enzyme is one or more of:
   i) UTP-dependent pyrophosphorylase;
   ii) UDP-glucose-dependent uridyl transferase; and
   iii) galactokinase.

75. The process of claim 75, wherein said one or more enzyme is two or more of:
   i) UTP-dependent pyrophosphorylase;
   ii) UDP-glucose-dependent uridyl transferase; and
   iii) galactokinase.

76. The process of claim 75, wherein said one or more enzyme is at least three of:
   i) UTP-dependent pyrophosphorylase;
   ii) UDP-glucose-dependent uridyl transferase; and
   iii) galactokinase.

77. The process of claim 74, wherein said one or more enzyme comprises UTP-dependent pyrophosphorylase.

78. The process of claim 74, wherein one or more enzyme comprises UDP-glucose-dependent uridyl transferase.

79. The process of claim 74, wherein one or more enzyme comprises UTP-dependent pyrophosphorylase and UDP-glucose-dependent uridyl transferase.

80. The process of claim 74, wherein said galactose is provided in the culture medium.

81. The process of claim 74, wherein said galactose is provided by exposing said cells or tissue to galactose-1-phosphate.

82. The process of claim 74, wherein said galactose is provided by exposing said cells or tissue to UDP-galactose.

83. The process of claim 74, wherein said cells or tissue are further exposed to a galactosidase that produces galactose from a galactose precursor.

84. The process of claim 74, wherein said cells or tissue are incubated in a culture medium containing one or more galactose precursor selected from: lactose, melibiose, raffinose, stachyose, verbascose, galactinol, galactose pentaacetate and galactose methyl galactoside, and wherein said medium further comprises an enzyme that converts said precursor to galactose.

85. The process of claim 74, wherein said cells or tissue are incubated in a culture medium containing one or more galactose derivative selected from: galactose-1-phosphate and UDP-galactose.

86. The process of claim 74, wherein said plant cells or tissue are tobacco, cotton, rape seed, potato, or maize plant cells or tissue.

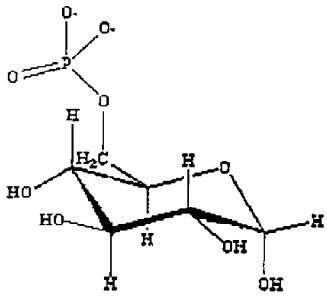
87. The process of claim 74, wherein said transforming further comprises transforming said cells or tissue with one or more heterologous nucleotide sequence of interest.

88. Transformed cells or tissue selected by the process of claim 74.

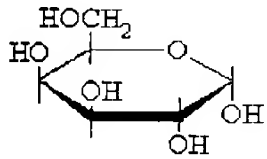
89. A transformed plant comprising cells or tissue selected by the process of claim 74.

Glycogen Biosynthesis & Galactose Pathway

View the 3-D version of this pathway.



Glucose-6-phosphate



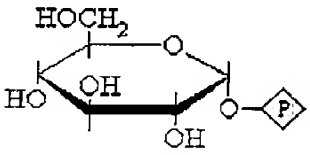
Galactose

Phosphoglucomutase

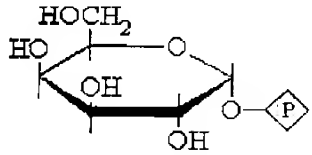
ATP

Galactokinase

ADP



Glucose-1-phosphate



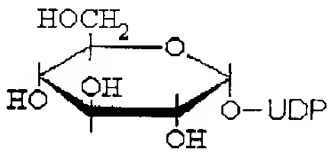
Galactose-1-phosphate

UTP

UDP-Glucose-pyrophosphorylase

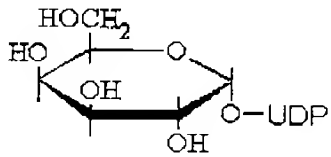
PP_i

UDP-Glc:Gal-1-P uridyltransferase

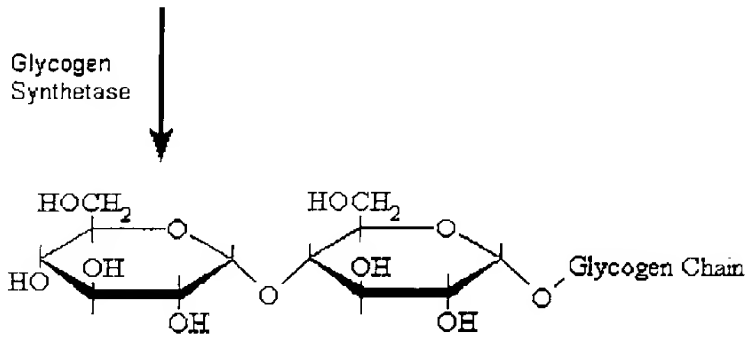


Uridine diphosphate glucose

UDP-Galactose Epimerase



Uridine diphosphate galactose



Glycogen

IUBMB Enzyme Nomenclature

EC 2.7.7.10

Common name: UTP-hexose-1-phosphate uridylyltransferase

Reaction: UTP + α-D-galactose 1-phosphate = diphosphate + UDP galactose

Other name(s): galactose-1-phosphate uridylyltransferase; galactose 1-phosphate uridylyltransferase; α-D-galactose 1-phosphate uridylyltransferase; galactose 1-phosphate uridyltransferase; UDPgalactose pyrophosphorylase; uridine diphosphate galactose pyrophosphorylase; uridine diphosphogalactose pyrophosphorylase

Systematic name: UTP:α-D-hexose-1-phosphate uridylyltransferase

Comments: α-D-Glucose 1-phosphate can also act as acceptor, but more slowly.

Links to other databases: BRENDA, EXPASY, KEGG, WIT, CAS registry number: 9016-11-9

References:

1. Isselbacher, K.J. A mammalian uridinediphosphate galactose pyrophosphorylase. *J. Biol. Chem.* 232 (1958) 429-444.

2. Kalckar, H.M. The role of phosphoglycosyl compounds in the biosynthesis of nucleosides and nucleotides. *Biochim. Biophys. Acta* 12 (1953) 250-264.

3. Lee, L., Kimura, A. and Tochikura, T. Purification and properties of UDP-glucose (UDP-galactose) pyrophosphorylase from *Bifidobacterium bifidum*. *J. Biochem. (Tokyo)* 86 (1979) 923-928. [Medline UI: 80049620]

4. Lobelle-Rich, P.A. and Reeves, R.E. Separation and characterization of two UTP-utilizing hexose phosphate uridylyltransferases from *Entamoeba histolytica*. *Mol. Biochem. Parasitol.* 7 (1983) 173-182. [Medline UI: 83219078]

[EC 2.7.7.10 created 1961]

IUBMB Enzyme Nomenclature

EC 2.7.7.12

Common name: UDPglucose-hexose-1-phosphate uridylyltransferase

Reaction: UDPglucose + α-D-galactose 1-phosphate = α-D-glucose 1-phosphate + UDPgalactose

Other name(s): uridyl transferase; hexose-1-phosphate uridylyltransferase; uridyltransferase; hexose 1-phosphate uridyltransferase

Systematic name: UDPglucose:α-D-galactose-1-phosphate uridylyltransferase

Links to other databases: BRENDA, EXPASY, GTD, KEGG, WIT, CAS registry number: 9026-21-5

References:

1. Kalckar, H.M., Braganca, B. and Munch-Petersen, A. Uridyl transferases and the formation of uridinediphosphogalactose. *Nature* 172 (1953) 1038 only.

2. Kurahashi, K. and Sugimura, A. Purification and properties of galactose 1-phosphate uridyl transferase from *Escherichia coli*. *J. Biol. Chem.* 235 (1960) 940-946.

3. Mayes, J.S. and Hansen, R.G. Galactose 1-phosphate uridyl transferase. *Methods Enzymol.* 9 (1966) 708-713.

4. Saito, S., Ozutsumi, M. and Kurahashi, K. Galactose 1-phosphate uridylyltransferase of *Escherichia coli*. II. Further purification and characterization. *J. Biol. Chem.* 242 (1967) 2362-2368. [Medline UI: 67165337]

5. Smith, E.E.B. and Mills, G.T. Uridyl transferase of mammary gland. *Biochim. Biophys. Acta* 18 (1955) 152 only.

[EC 2.7.7.12 created 1961]

IUBMB Enzyme Nomenclature

EC 2.7.1.6

Common name: galactokinase

Reaction: ATP + D-galactose = ADP + α-D-galactose 1-phosphate

Other name(s): galactokinase (phosphorylating); ATP:D-galactose-1-phosphotransferase

Systematic name: ATP:D-galactose 1-phosphotransferase

Comments: D-Galactosamine can also act as acceptor.

Links to other databases: BRENDA, EXPASY, GTD, KEGG, WIT, CAS registry number: 9030-53-9

References:

1. Cardini, C.E. and Leloir, L.F. Enzymic phosphorylation of galactosamine and galactose. *Arch. Biochem. Biophys.* 45 (1953) 55-64.

2. Neufeld, E.F., Feingold, D.S. and Hassid, W.Z. Phosphorylation of D-galactose and L-arabinose by extracts from *Phaseolus aureus* seedlings. *J. Biol. Chem.* 235 (1960) 906-909.

3. Wilkinson, J.F. The pathway of the adaptive fermentation of galactose by yeast. *Biochem. J.* 44 (1949) 460-467.

[EC 2.7.1.6 created 1961]

IUBMB Enzyme Nomenclature

EC 5.1.3.2

Common name: UDPglucose 4-epimerase

Reaction: UDPglucose = UDPgalactose

Other name(s): UDPgalactose 4-epimerase; uridine diphosphoglucose epimerase; galactowaldenase; UDPG-4-epimerase; uridine diphosphate galactose 4-epimerase; uridine diphospho-galactose-4-epimerase; UDP-glucose epimerase; UDP-galactose 4-epimerase; 4-epimerase; UDPG-4-epimerase; uridine diphosphoglucose 4-epimerase; uridine diphosphate glucose 4-epimerase; UDP-D-galactose 4-epimerase

Systematic name: UDPglucose 4-epimerase

Comments: Requires NAD. Also acts on UDP-2-deoxyglucose.

Links to other databases: BRENDA, EXPASY, GTD, KEGG, WIT, CAS registry number: 9032-89-7

References:

1. Leloir, L.F. Enzymic isomerization and related processes. *Adv. Enzymol. Relat. Subj. Biochem.* 14 (1953) 193-218.

2. Maxwell, E.S. and de Robichon-Szulmajster, H. Purification of uridine diphosphate galactose-4-epimerase from yeast and the identification of protein-bound diphosphopyridine nucleotide. *J. Biol. Chem.* 235 (1960) 308-312.

3. Wilson, D.B. and Hogness, D.S. The enzymes of the galactose operon in *Escherichia coli*. I. Purification and characterization of uridine diphosphogalactose 4-epimerase. *J. Biol. Chem.* 239 (1964) 2469-2481.

[EC 5.1.3.2 created 1961]